UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

November 21, 2007

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No. 10324-93
DP Barcode: D342461

To: Velma Noble, PM 31/ Tracy Lantz
Regulatory Management Branch I
Antimicrobials Division (7510C)

From: Wallace Powell, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510C)

Through: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510C)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

Applicant: Mason Chemical Company

ACTIVE INGREDIENTS	% by wt.
(Code 069104) Alkyl* dimethyl benzyl ammonium chloride *(60%C14, 30%C16, 5%C18, 5%C12)	2.25
(Code 069154) Alkyl* dimethyl ethylbenzyl ammonium chloride *(68%C12, 32%C14)	2.25

BACKGROUND

In support of a label amendment for the subject product MAQUAT 64 PD, EPA Reg. No. 10324-93, the applicant has submitted studies for acute oral toxicity, acute dermal toxicity, skin irritation, and skin sensitization. The studies were initially reviewed for Product Science Branch (PSB) by CSC Systems & Solutions LLC. The reviews are attached to this memorandum. They may have been modified after a brief secondary review by PSB. An eye irritation data waiver request was also submitted (MRID 471861-04). The Cite-all option was chosen for acute inhalation toxicity.

RECOMMENDATIONS

The submitted **acute oral toxicity** study is unacceptable. The data are not sufficient to place the LD_{50} for the females in an acute Toxicity Category. Given the results obtained, at least one additional dose level was needed in the females.

Note: The submitted acute oral toxicity study used the traditional Guideline 401 method. (This method uses five animals per sex per dose level and is not characterized by the stepwise dosing progression of the Up-and-Down method.) This method is no longer accepted by Office of Pesticide Programs for new studies. If a new study is conducted (rather than citing a previously reviewed study), it should be conducted using one of the currently accepted methods. These methods are found at http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised .

The submitted **acute dermal toxicity** study and **skin irritation** study are acceptable.

The Cite-all method of support proposed for the **acute inhalation toxicity** data requirement is unacceptable. The submitted data matrix indicates Cite-all but fails to specify an EPA-registered product on which EPA can conduct a similarity determination. However, the acute inhalation toxicity data requirement apparently was waived previously. Because of this and because no acute inhalation-related labeling changes are proposed, no acute inhalation data need be required at this time, though the data requirement will need to be addressed anew at the time of Reregistration.

The **eye irritation** waiver request is acceptable, based on the skin reactions in the submitted acute dermal toxicity study, as also affirmed by the written opinion from the testing laboratory.

The submitted **skin sensitization** study is unacceptable but potentially upgradable:

1. No erythema reactions were reported for the induction phase of the main study. Graded reactions should be reported for each animal, in order to confirm the adequacy of the induction concentration. (The reported results of the Irritation Screen are helpful in this regard but in PSB's opinion are insufficient.)

2. No erythema reactions were reported for the naïve control group in the positive control study.

Acute toxicity profile based on newly submitted data support only:

Study	MRID	Toxicity Category	Status
Acute Oral Toxicity	471861-02	---	Unacceptable
Acute Dermal Toxicity	471861-01	III	Acceptable
Acute Inhalation Toxicity	Cite-all	---	Unacceptable
Primary Eye Irritation	Waiver request	I	Acceptable
Primary Skin Irritation	471861-05	III	Acceptable
Dermal Sensitization	471861-03	---	Unacceptable

LABELING

A review of the first-aid and human-hazard precautionary statements cannot be completed until the acute oral toxicity, acute inhalation toxicity, and skin sensitization data requirements have been satisfied. However, the following comments apply.

The proposed deletion of the "harmful if swallowed" statement is unacceptable without acceptable supporting data on acute oral toxicity.

Although the statement, "Wash thoroughly with soap and water after handling," has not been altered since the last Accepted label, note that it should be expanded to read: "Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco," as per the *Label Review Manual.*

The proposed deletion of the "[causes] skin burns" statement is acceptable.

The proposed change in the "do not get on skin" precaution to read, "Avoid contact with skin," is acceptable.

The First Aid section is acceptable.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)

Product Manager: 31
MRID No.: 471861-02

Reviewer: W. Powell
Study Completion Date: November 10, 2005
Report No.: 05-087-3

Testing Laboratory: Tox Monitor Laboratories, Inc., Oak Park, IL
Author: Michael Kukulinski, B.S., L.A.T.G.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study was conducted in accordance with U.S. EPA GLP Standards, 40 CFR 160.

Test Material: Maquat 64 PD
Batch #: 1621-194 / Clear liquid

Dosage: 5,000 mg/kg (administered neat)

Species: 10 Rats; Sprague-Dawley derived, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (6-10 weeks old)
Weight: 200-235 grams
Source: Harlan Sprague Dawley, Indianapolis, IN
Housing: Temperature Range: 64-79 °F (i.e., 17.8-26.1°C)
Humidity Range: 30-70%
Photoperiod: [Information regarding light/dark cycle not provided]
Acclimation: At least 5 days

Conclusion:

1. **LD_{50} (mg/kg)**: Male Rats: >5,000 mg/kg
   Female Rats: Undetermined

2. **Toxicity Category**: Undetermined.
   Classification: **Unacceptable**, based on the first *Deviation* listed below

Procedure (Deviations from 870.1100):

- Given the results obtained, at least one additional dose level should have been used in the females. The data are not sufficient to place the LD_{50} for the females in an acute Toxicity Category.
- The guidelines state that young adult animals should be between 8 and 12 weeks old at the commencement of dosing. The animals used in this study were between 6 and 10 weeks old.
- The lower and upper limits of the animal housing temperature range were outside the recommended temperature range of 22±3°C.

- The guidelines state that the sequence of animal housing artificial lighting should be 12 hours light/ 12 hours dark. The laboratory reported only that the test animals were housed in a light controlled room.
- The guidelines state that animals should be observed individually at least once during the first 30 minutes after dosing. The first observation reported by the laboratory for each animal was at 1 hour after dosing.

Results:

The administration of the test substance by oral gavage at a dose of 5,000 mg/kg body weight to male and female rats produced 30% mortality in the ten test animals.

Reported Mortality

Dosage (mg/kg)	Number Deaths / Number Tested		
	Male	Female	Total
5,000	0 / 5	3 / 5	3 / 10

Observations:

30% mortality occurred during the 14-day observation period. The most prevalent gross pharmacotoxic observations following test substance administration included hunched posture, hypoactivity, and piloerection. Facial staining was observed in one animal.

Body weight gain for all surviving test animals was unaffected by the administration of the test substance. All surviving test animals gained weight throughout the study.

Gross Necropsy Findings:

Gross external necropsy findings of two of the three test animals that died during the course of the study revealed staining around the nose/ mouth or anal area. Gross internal necropsy findings of the three test animals that died during the course of the study were primarily confined to the gastrointestinal system. Observations included stomach and GI tracts distended with gas and/ or fluid, and stomachs and GI tracts red in color. One animal also showed mottling of the liver.

External and internal necropsy findings of test animals surviving to Day 14 revealed no gross abnormalities.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 31
MRID No.: 471861-01

Reviewer: W. Powell
Study Completion Date: November 16, 2005
Report No.: 05-087-4

Testing Laboratory: Tox Monitor Laboratories, Inc., Oak Park, IL
Author: Michael Kukulinski, B.S., L.A.T.G.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study was conducted in accordance with U.S. EPA GLP Standards, 40 CFR 160.

Test Material: Maquat 64 PD
Batch #: 1621-194 / Clear liquid

Dosage: 2,000 mg/kg (administered neat)

Species: 10 Rabbits; New Zealand, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (12 weeks old)
Weight: 2.08-2.47 kilograms
Source: Kuiper Rabbitry, Gary, IN
Housing: Temperature: 61-72°F (i.e., 16.1-22.2°C)
Humidity: 30-70%
Photoperiod: [Information regarding light/dark cycle not provided]
Acclimation: At least 5 days

Summary:

1. **LD_{50} (mg/kg)**: Male and Female Rabbits: >2,000 mg/kg

2. **The estimated dermal LD_{50} is** greater than 2,000 mg/kg in male and female rabbits.

3. **Toxicity Category: III** **Classification: Acceptable**

Procedure (Deviations from 870.1200):

- The lower limit of the animal housing temperature range was below the recommended temperature range of 20±3°C.
- The guidelines state that, where animal housing lighting is artificial, the sequence should be 12 hours light/ 12 hours dark. The laboratory reported only that the test animals were housed in a light controlled room.

Results:

The administration of the test substance by dermal application at a dose of 2,000 mg/kg body weight to male and female rabbits produced no mortality, indicating that the dermal LD_{50} of the sample is greater than 2,000 mg/kg body weight.

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
2,000	0 / 5	0 / 5	0 / 10

Observations:

No mortality occurred during the 14-day observation period. All ten test animals exhibited erythema, edema, eschar, and chemical burns at the application site during the 14-day observation period.

All ten test animals exhibited erythema and edema at the application site on Day 1. Nine of the ten test animals also exhibited chemical burns on Day 1. The other test animal did not develop chemical burns until Day 3. All animals were free of erythema by Day 4. Nine of the ten test animals exhibited eschar on Day 3. The other test animal (the same animal that had delayed development of chemical burns) did not develop eschar until Day 4. All ten test animals exhibited edema, eschar, and chemical burns at the application site at the end of the 14-day observation period.

Body weight gain for all ten test animals was affected by the administration of the test substance. Body weight loss was noted in eight of ten test animals at Day 7. Two animals continued to lose weight between Days 7 and 14. Only four animals' weight on Day 14 exceeded the initial body weights.

Gross Necropsy Findings:

Gross external necropsy findings revealed eschar and chemical burns at the application site for all ten test animals. Internal necropsy findings revealed no gross abnormalities.

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 31
MRID No.: 471861-05

Reviewer: W. Powell
Study Completion Date: January 26, 2006
Report No.: 05-087-2

Testing Laboratory: Tox Monitor Laboratories, Inc., Oak Park, IL
Author: Michael Kukulinski, B.S., L.A.T.G.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study was conducted in accordance with U.S. EPA GLP Standards, 40 CFR 160.

Test Material: Maquat 64 PD
Batch #: 1621-194 / Clear liquid

Dosage: 0.5 mL (applied as received)

Species: 6 Rabbits; New Zealand, albino
Sex: 3 Males and 3 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8-10 weeks old)
Weight: 2.27-2.73 kilograms
Source: Kuiper Rabbitry, Gary, IN
Housing: Temperature: 61-72°F (i.e., 16.1-22.2°C)
Humidity: 30-70%
Photoperiod: [Information regarding light/dark cycle not provided]
Acclimation: At least 5 days

Summary:

1. **Toxicity Category: III**

2. **Classification: Acceptable**

Procedure (Deviations from 870.2500):

- The guidelines state that irritation responses should be scored with 30-60 minutes after patch removal. The first reading reported by the laboratory for each animal was at 4.5 hours after patch removal.

Results:
Within 4.5 hours of patch removal, all six treated sites exhibited erythema, with four being more affected than the other two. The overall incidence and severity of irritation increased thereafter, peaking at 168 hours when two showed moderate to severe erythema, and the other four showed only well-defined erythema. Within 4.5 hours of patch removal, all six treated sites also exhibited edema, with one animal being less affected than the others. This decreased over time, and all animals were free of edema by 336 hours. All animals were free of dermal irritation by 504 hours.

The maximum primary skin irritation score was 3.5 at the 4.5-hour observation. The Primary Dermal Irritation Index for the test substance was calculated to be 3.29. [Scores for observations made during the first 4.5, 24, 48, and 72 hours were used in this calculation.] Under the conditions of this study, Maquat 64 PD is classified as moderately irritating to the skin.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
4.5 hours	6 / 6	6 / 6
24 hours	6 / 6	6 / 6
48 hours	6 / 6	6 / 6
72 hours	6 / 6	4 / 6
168 hours	6 / 6	4 / 6
336 hours	5 / 6	0 / 6
504 hours	0 / 6	0 / 6

Individual Skin Irritation Scores

Animal No.	Sex	Erythema / Edema						
		Time After Patch Removal						
		4.5 hrs	24 hrs	48 hrs	72 hrs	168 hrs	336 hrs	504 hrs
876	**F**	1 / 2	1 / 2	2 / 1	2 / 1	2 / 1	2 / 0	0 / 0
877	**F**	2 / 2	2 / 2	2 / 1	2 / 1	3 / 1	2 / 0	0 / 0
878	**F**	1 / 1	1 / 1	2 / 1	2 / 0	2 / 0	0 / 0	0 / 0
884	**M**	2 / 2	2 / 1	2 / 1	2 / 0	2 / 0	2 / 0	0 / 0
885	**M**	2 / 2	2 / 2	2 / 2	2 / 2	3 / 1	2 / 0	0 / 0
886	**M**	2 / 2	2 / 2	2 / 2	2 / 2	2 / 1	2 / 0	0 / 0
Total		10 / 11	10 / 10	12 / 8	12 / 6	14 / 4	10 / 0	0 / 0
Mean		1.67 / 1.83	1.67 / 1.67	2.0 / 1.33	2.0 / 1.0	2.33 / 0.67	1.67 / 0	0 / 0

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(BUEHLER METHOD)

Product Manager: 31
MRID No.: 471861-03

Reviewer: W. Powell
Study Completion Date: December 9, 2005
Report No.: 05-087-5

Testing Laboratory: Tox Monitor Laboratories, Inc., Oak Park, IL
Author: Michael Kukulinski, B.S., L.A.T.G.

Quality Assurance (40 CFR §160.12): A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study was conducted in accordance with U.S. EPA GLP Standards, 40 CFR 160.

Test Material: Maquat 64 PD
Batch #: 1621-194 / Clear liquid

Positive Control Material: 1-Chloro-2,4-Dinitrobenzene
(Historical data – completed June 8, 2005)

Species:	32 Guinea pigs; Hartley, albino
Sex:	Range-Finding: 2 Males
	Test Group: 20 Males
	Naïve Control Group: 10 Males
Age:	Young adult (specific age not reported)
Weight:	300-500 grams at the start of the study
Source:	Kuiper Rabbitry, Gary, IN
Housing:	Temperature: 64-79°F (i.e., 17.8-26.1°C)
	Humidity: 30-70%
	Photoperiod: 12-hour light/dark cycle
Acclimation:	At least 5 days

Method: Buehler Technique Guinea Pig Sensitization Protocol

Summary:

1. Based on these findings and on the evaluation system used, Maquat 64 PD did not appear to be a contact sensitizer.

2. **Classification**: **Unacceptable** (but potentially upgradable), based on the first two *Deviations* listed below.

Procedure (Deviations from 870.2600):

- No erythema reactions were reported for the induction phase of the main study. Graded reactions should be reported for each animal in order to confirm the adequacy of the induction concentration. (The reported results of the Irritation Screen are helpful in this regard but in PSB's opinion are insufficient.)

- No erythema reactions were reported for the naïve control group in the positive control study.
- The upper limit of the animal housing temperature range was above the recommended temperature range of 20±3°C.

Procedure:

Irritation Screening (Pilot): The irritation phase had the purpose of determining the irritation potential of the test substance. The irritation potential of the test substance at levels of 20%, 15%, 10%, and 5% were evaluated in one group of two animals. Four levels of test substance were evaluated per animal. Dilutions of the test substance were formulated w/w in distilled water. The position of the different concentrations of the test substance on the animals was varied to adjust for possible site-to-site variation in response.

Preparation of Animals and Test Substance Administration: The day prior to test substance exposure, the hair was removed from each of the animals' backs using a small animal clipper. Closed patches were applied to the animals in the following manner: A 0.4 mL quantity of each test preparation, diluted to a 15% concentration with distilled water, was applied directly into a 25 mm Hilltop Chamber. The animals were held gently, and the chambers were applied as quickly as possible to the clipped left shoulder. The chambers were secured with Micropore tape and further secured with Kendall adhesive tape. Approximately six hours later, the tape and chambers were removed. The day following the irritation exposure, all animals were scored for erythema. The grading was repeated the following day.

Based upon the irritation screen results, the test substance was dosed as 15% concentration in distilled water for the induction phase of the study, and 10% concentration in distilled water, the highest non-irritating concentration, for the challenge phase of the study.

Induction Phase: The purpose of this phase was to dermally expose the animals to the test substance so that, if the material is a sensitizer, the physiological process required to ultimately allow the generation of an immunological response can be initiated. The left shoulder of each test animal was clipped with a small animal clipper the day before exposure. The animals were held gently and the chambers were applied as described above. Two additional induction doses were conducted following the same procedure, at weekly intervals. After the last induction exposure, the animals were left untreated for two weeks (14 days) before primary challenge.

Challenge Phase: The purpose of this phase was to investigate the elicitation of response to the test substance. The test animals, which had three previous exposures to the test substance at appropriate intervals, were exposed to the test substance in the challenge phase, fourteen days after the last induction exposure. The same exposure procedure as for the "Induction Phase" was used, except the Hilltop Chambers were applied to a skin site that had not been previously exposed and the product was diluted to a 10% concentration in distilled water. [Each animal received a single chamber of the test substance at Site 3.] The day following the primary challenge exposure, all animals were scored for erythema. The grading was repeated the following day as well. For reporting purposes, the first and second gradings were designated as 24 and 48 hours readings, respectively.

Test System Justification: The procedures used in this study were validated using 1-Chloro-2,4-Dinitrobenzene as a positive control substance. Recent testing was completed on June 8, 2005.

Results:

Sensitization Response Indices (Erythema)

	Incidence of Positive Response[1]		**Severity[2]**	
	Hours		**Hours**	
	24	**48**	**24**	**48**
Test Animals – Challenge	2 / 20	0 / 20	0.05	0.0
Naïve Control Animals – Challenge	1 / 10	0 / 10	0.05	0.0

[1]The number of animals in each group showing a specific response (greater than 0.5) at either the first or second readings.
[2]The sum of the test grades divided by the total number of animals tested in a given group, determined separately for both the first and second readings.

Test Animal Group Skin Reaction Scores

Treatment Phase	Induction						Challenge	
	1		2		3			
Concentration	15%		15%		15%		10%	
Hours[1]	24	48	24	48	24	48	24	48
Animal No. / Sex								
Test Group								
634 / M	*	*	*	*	*	*	0	0
635 / M	*	*	*	*	*	*	0	0
636 / M	*	*	*	*	*	*	0	0
637 / M	*	*	*	*	*	*	0	0
638 / M	*	*	*	*	*	*	0	0
639 / M	*	*	*	*	*	*	0	0
640 / M	*	*	*	*	*	*	0	0
641 / M	*	*	*	*	*	*	0.5	0
642 / M	*	*	*	*	*	*	0	0
643 / M	*	*	*	*	*	*	0	0
644 / M	*	*	*	*	*	*	0	0
645 / M	*	*	*	*	*	*	0	0
646 / M	*	*	*	*	*	*	0	0
647 / M	*	*	*	*	*	*	0	0
648 / M	*	*	*	*	*	*	0	0
649 / M	*	*	*	*	*	*	0	0
650 / M	*	*	*	*	*	*	0	0
651 / M	*	*	*	*	*	*	0	0
652 / M	*	*	*	*	*	*	0.5	0
653 / M	*	*	*	*	*	*	0	0
Naïve Control Group								
654 / M	---	---	---	---	---	---	0.5	0
655 / M	---	---	---	---	---	---	0	0
656 / M	---	---	---	---	---	---	0	0
657 / M	---	---	---	---	---	---	0	0
658 / M	---	---	---	---	---	---	0	0
659 / M	---	---	---	---	---	---	0	0
660 / M	---	---	---	---	---	---	0	0
661 / M	---	---	---	---	---	---	0	0
662 / M	---	---	---	---	---	---	0	0
663 / M	---	---	---	---	---	---	0	0

[1] Hours after induction dose.
*Not reported.